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Acceleration of Precipitation Formation in Peach Juice Induced by High-Pressure Carbon Dioxide

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Peach juice was treated by high-pressure carbon dioxide (HPCD). HPCD-induced acceleration of precipitation formation in peach juice was observed. Particle size distribution (PSD), pH, ζ-potential, protein and total phenols, pectin methylesterase (PME) activity, pectin and calcium, and viscosity in juice, contributing to the precipitation formation, were studied. HPCD resulted in a significant alteration of juice PSD pattern; the acceleration of the precipitation formation concurred with this alteration. A significant decrease of protein and a declining trend of total phenols were obtained, the contents of pectin and calcium were not changed, and the difference in PME activity in juice was not significant after HPCD. HPCD led to higher increase in juice viscosity, whereas pH and the absolute value of ζ-potential declined during HPCD. These results suggested that the pH and absolute value of ζ-potential declines induced the coagulation of protein and decrease of particle charge, responsible for the acceleration of the precipitation formation.

KEYWORDS: Particle size distribution; pH; ζ-potential; pectin methylesterase; viscosity; turbidity

INTRODUCTION

In cloudy juice products, the cloudy mass related to the flavor, turbidity, and color of the juice provides a significant quality attribute in processing. This mass can be caused by a colloidal suspension in which the continuous medium refers to a solution of pectin, sugars, and organic acids, etc., and the dispersed matter is mainly formed by the cellular tissue comminuted during fruit processing (*1*). If the particles in the cloudy mass adhere together and form flocs, they may precipitate due to gravity and make the solution clear. Filippi et al. (*1*) noted that the formation of the dense flocs can be irreversible. According to the classical DLVO theory, the stability of the colloidal particles depends on the balance between the attractive van der Waals forces and repulsive electrostatic forces; the latter can be reduced by the presence of counterions in the system (*2*, *3*). In juice solution, the presence of the mobile cations can decrease the electrostatic repulsion and promote aggregation by screening the negative charges on the surface of the particles and, consequently, destroy the stability of the solution (*2*). Moreover, much work focused on pectin methylesterase (PME, EC 3.1.1.11), which was considered to be the main cause for loss of cloud stability (*4*). PME can act on pectin to yield acidic low-methoxy pectin, which can cross-link with the polyvalent cations, such as Ca^{2+}, to form insoluble pectate precipitates (*4*).

Thermal processing, a most common and least expensive method in the food industry, is frequently used to inactivate PME for preserving juice cloud (*5*). However, this method can be detrimental to the organoleptic and nutritional qualities of the juice (*6*). Moreover, as consumers prefer minimally processed and fresh-tasting food products, the application of nonthermal technologies such as high-pressure carbon dioxide (HPCD), which has noticeable inactivation effects on microorganisms (*7*−*9*) and enzymes (*5*, *6*, *10*−*12*) in fruit juices, became popular in the past decade. However, destabilization of cloudy juices by HPCD has been known (*13*, *14*), which could reduce the appearance quality of the juice. Significant increase in the particle size of carrot juice by HPCD was observed in a previous study (*13*). Park et al. (*14*) have already reported that the rapid destabilization of cloudy juice is not correlated with PME action, but the relevant details are few in the literature. To better understand the acceleration of the precipitation formation and the role of the HPCD, the focus of the present work is to analyze factors including particle size distribution, pH, and ζ-potential, protein and total phenols, pectin methylesterase activity, pectin and calcium, and viscosity in juice, contributing to the precipitation formation, and to compare the effects of HPCD and heat on the stability of the peach juice.

MATERIALS AND METHODS

Materials. Peaches (cultivar No. 24 Beijing) were purchased from Beijing Guangyuan Yanwei Agricultural Science and Technology Co., Ltd., belonging to the practice base of China Agricultural University. Apple pectin (DE 70−75%) was obtained from Andre Co. (Shangdong, China). Bovine serum albumin was purchased from Amersco (Beijing, China). D-(+)-Galacturonic acid and 3-hydroxybiphenyl were obtained from Sigma-Aldrich Chemical Co. (Beijing, China). All other chemicals were of analytical grade.

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Preparation of Peach Juice. Peach halves were pitted and sliced by hand, juiced with a screw juice extractor (GT6G7, Zhejiang Light Industry Machinery Plant, Zhejiang, China), and filtered with four layers of cheesecloth. As an antibrowning agent, 0.15% (w/w) of L-ascorbic acid was added into slices before juicing. The resulting juice was packed into 250 mL glass bottles and stored at −18 °C. Prior to use, it was thawed in an ambient running water bath at 25 °C for about 2 h. Removing the coarse pulp by centrifugation (1500*g*, 15 min, 4 °C), to ensure even distribution of pulp in the juice, was necessary before HPCD.

HPCD Process System. The HPCD system was described by Liao et al. (*7*). The stainless steel pressure vessel with a volume of 850 mL was designed to withstand a pressure of 50 MPa. The vessel temperature was maintained by a THYS-15 thermostatic bath (Ningbo Tianheng Instrument Factory, Zhejiang, China). An XMTA-7512 temperature controller (Yuyao Temperature Meter Factory, Zhejiang, China) was used to monitor the temperature with two thermocouples. One thermocouple was fixed in the vessel lid to monitor the CO_2 temperature in the upper part of the vessel, and the other was placed at the middle wall of the vessel to monitor the temperature of the peach juice in the vessel. A 2TD plunger pump (Huaan Supercritical Fluids Extraction Co. Ltd., Jiangsu, China) with a maximum pressure of 50 MPa and a maximum flow rate 50 L/h was used to pressurize the vessel. A DBY-300 pressure transducer (Shanxi Qingming Electronic Group Corp., Shanxi, China) was fixed in the vessel lid to monitor the vessel pressure. All of the temperature and pressure data were displayed on a control panel. All parts of the system exposed to high pressure were made of stainless steel. The vessel had gastight connections to the gas inlet and outlet and to the fluid sample inlet and outlet. The vessel lid could be sealed by screws during HPCD processing. A 2XZ-4 vacuum pump (Huangyan Qiujing Vacuum Pump Factory, Zhejiang, China) was connected to the vessel for evacuating the air in the vessel and building the vacuum state of the vessel. The peach juice samples were drawn into the vessel by negative pressure in the vessel. A DL-CJ-1F biohazard containment unit (Donglian Electron Technology Co., Ltd., Haerbin, China) was used for aseptic operation. Commercially available CO_2 of 99.5% purity was purchased from Beijing Jingcheng Co. (Beijing, China) and was passed through an active carbon filter before entering the pressure vessel.

Processing of Peach Juice Using the HPCD System. After being rinsed and sanitized, the pressure vessel was heated to the required temperature and was evacuated. A 400 mL sample of peach juice was drawn to the evacuated pressure vessel by opening the sample inlet valve. The sample inlet valve was then closed, and the CO_2 inlet valve was opened. The vessel was pressurized by the plunger pump to the required pressure level, and the required pressure was held for the required treatment time. Then the depressurization was performed by opening the pressure relief valve at the CO_2 outlet on the pressure vessel. The compression time was about 5 min, the depressurization time between 4 and 5 min, and the temperature of the product decreased due to the Joule−Thomson cooling effect. Equilibration of the temperature was achieved in about 5 min due to the use of a thermostatic bath. After HPCD, the treated samples were filled into a sterile bottle through a sample outlet valve. The HPCD system was rinsed and washed three times with deionized water after processing of each sample.

The treatment parameters applied in this study were as follows: pressure, 30 MPa; temperature, 55 °C; treatment time, 30 s and 10, 40, and 60 min. The parameters of pressure and time were selected on the basis of the Liao et al. (*7*) study and were effective in inactivating microbes.

Heat Treatment of Peach Juice. Peach juice was placed in a stainless steel cup (80 × 100 mm, wall thickness = 1.15 mm) and heated to 90 °C at the center of juice for 1 min in a water bath; the time for temperature rising was about 5 min. The heat-treated juice was cooled to 15 °C after 5 min in an ice−water bath and then stored at 4 °C for various measurements.

Estimation of Precipitation Formation Time. The time of precipitaiont formation was estimated immediately after HPCD or heat, until precipitation appeared. In the case of untreated juice, it was timed from removing the coarse pulp by centrifugation until precipitation appeared.

Determination of pH. pH was measured at 20 °C with a Thermo Orion 868 pH-meter (Thermo Fisher Scientific, Inc., Pittsburgh, PA), which was calibrated with pH 4.0 and 7.0 buffer.

Determination of Turbidity. The method of Arreola et al. (*15*) with some modifications was used to measure turbidity. Samples were centrifuged at 4200*g* for 15 min (TGL-16G-A refrigerated centrifuge, Anting Apparatus Co., Shanghai, China). The supernatant was poured into 3 mL cuvettes and placed in a spectrophotometer (Lingguang, Shanghai, China) calibrated with distilled water, and absorbance at 660 nm was measured.

Determination of Particle Size Distribution (PSD). The PSD of juice was determined by an LS 230 particle size analyzer (Beckman Coulter, Inc., Brea, CA) (*13*). The system used a laser light with a wavelength of 750 nm to measure particles from 0.4 to 2000 μm by light diffraction. Fourier optics collected the diffracted light, and the PSD was calculated by using the Fraunhofer model.

First, distilled water from a tank was pumped into a sample cell at the speed of approximately 8 L/min until the cell was full. The peach juice was added into the cell using a pipet and mixed with distilled water. Particles in juice were dispersed and suspended in distilled water. When the obscuration percentage increased from 0 to 10%, the measurement was performed. Data obtained were analyzed using software LS v 3.29. Size distributions (volume fractions against particle size) were calculated. Volume mean diameter d_{43} and surface mean diameter d_{32} were calculated for all samples.

Determination of Viscosity. The dynamic viscosity of peach juice was determined using an AR550 rheometer (TA Instruments, Waters Co., Ltd., Surrey, U.K.) with a conical end concentric cylinder (stator radius = 15.00 mm, rotor radius = 14.00 mm, immersed height = 42.00 mm, gap = 5920 μm) (*13*).

A 19.6 mL amount of juice was applied at each measurement with 25 ± 0.1 °C controlled by circulating water in a thermostatic system. Viscosities were obtained in the shear rate range between 6 and 252 s^{-1}.

Pectin Methylesterase Assay. PME activity was determined by measuring the release of acid as a function of time at pH 7.5 and 30 °C according to the method described by Sampedro et al. (*16*) with some modifications. The reaction mixture consisted of 5 mL of peach juice and 60 mL of 1% apple pectin solution (DE 75%) containing 0.1 M NaCl. Before injection, the pectin solution was adjusted to pH 7.5. During hydrolysis at 30 °C, pH was maintained at 7.5 by adding 0.01 N NaOH using an automatic pH-stat titrator (Metrohm, Switzerland). The consumption of NaOH was recorded for a 15 min reaction period. The PME activity unit (U) is defined as micromoles of acid produced per minute at pH 7.5 and 30 °C.

Determination of Total Phenols, Protein, and Calcium Contents. The peach juice was centrifuged at 23800*g* and 4 °C for 30 min to separate the serum and precipitate. The serum was collected and analyzed for total phenols and protein content. The precipitate was then thoroughly mixed with deionized water and centrifuged (10000*g*, 10 min, 4 °C) again. The supernatant was discarded. This step was repeated until the supernatant solution contained no color or no further visual dilution in color was observed. Then the washed precipitate was lyophilized and kept in a tight desiccator until analysis (*17*).

Twenty milligrams of precipitate was dissolved in 10 mL of deionized water, and the solution was thoroughly mixed by vortexing for analysis. Total phenols of serum and precipitation were determined according to the modified Folin−Ciocialteu procedure as described previously (*17*). Two milliliters of 10-fold diluted Folin−Ciocalteu reagent was added to a 0.2 mL sample; 0.8 mL of 7.5% (w/v) Na_2CO_3 solution was added, and the mixture was mixed by vortexing. After 1 h, the absorbance was measured at 765 nm. The total phenols content was expressed as gallic acid equivalents in milligrams per milligram of precipitant (%). Protein contents of the serum and precipitate were determined according to the method of Bradford (*18*) using bovine serum albumin as standard.

The calcium content of serum was analyzed with a Z-2000 atomic absorption spectrophotometer (Hitachi, Japan) according to method GB/T 5009.92-2003. Samples were digested in concentrated acid (HNO_3/$HClO_4$, 4:1) and then diluted with 20 g/L $LaCl_3$ solution. The dilution solution was analyzed for calcium with an atomic absorption spectrophotometer.

Determination of Pectin Content. The alcolol-insoluble residue (AIR) of peach juice was obtained as described by Fraeye et al. (*19*) with some modification. Ten grams of peach juice was mixed in 100 mL of 95% ethanol. After boiling for 1 h, the insoluble solids were collected by centrifugation at 9000*g* for 15 min. This procedure was continued until a clear residue was obtained. The final extract was collected using a Buchner funnel and washed with 95% ethanol. The residue was dissolved in 50 mL of deionized water and stored at −20 °C until analysis.

Pectin content was determined according to the method described by the colorimetric method (*20*) with a spectrophotometer (UV-762,

Table 1. Characteristics of Peach Juice Treated by HPCD[a]

	precipitation formation time (h)	d_{43} (μm)	d_{32} (μm)	pH	ζ-potential (mV)	Ca (mg/L)
untreated	60 ± 3	2.15 ± 0.01d	1.24 ± 0.01d	3.82 ± 0.02a	−6.76 ± 0.36a	31.8
30 s	6 ± 1	28.28 ± 0.45a	6.80 ± 0.14a	3.83 ± 0.01a	−6.68 ± 0.25a	28.7
10 min	5 ± 1	22.31 ± 0.51b	4.53 ± 0.08b	3.83 ± 0.02a	−6.56 ± 0.34a	31.6
40 min	5 ± 1	19.31 ± 1.35bc	3.41 ± 0.01c	3.78 ± 0.03a	−6.29 ± 0.25a	34.65
60 min	7 ± 1	17.65 ± 1.53c	3.19 ± 0.37c	3.80 ± 0.03a	−6.92 ± 0.26a	31.8
heat (90 °C, 1 min)	60 ± 2	3.66 ± 0.24d	1.19 ± 0.03d	3.82 ± 0.02a	−6.54 ± 0.20a	34.25

[a] Peach juices were treated by HPCD at 30 MPa and 55 °C for 30 s, 10 min, 40 min, and 60 min, and heat at 90 °C for 1 min.

Lingguang, Shanghai, China). The pectin concentration was expressed as D-galacturonic acid equivalents in percentage.

Determination of ζ-Potential. ζ-potential has been used to assess the stability of colloidal systems because it is a very good index of the magnitude of colloidal electrostatic repulsive forces (*21*). Prior to determination, peach juice was centrifuged (4200*g*, 15 min, 4 °C) to remove big particles. A Delsa Nano instrument (Beckman Coulter) was used to determine the ζ-potential values with a flow cell at room temperature.

Statistical Analysis. Analysis of variance (ANOVA) was carried out by using the software Microcal Origin 7.5 (Microcal Software, Inc., Northampton, MA). ANOVA tests were carried out for all experimental runs to determine significance at the $\alpha = 0.05$ level. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Acceleration of Precipitation Formation and PSD Analysis. An acceleration of the precipitation formation was observed as opposed to the untreated and heat-treated juices. As shown in **Table 1**, the time required for the observed precipitation formation in the HPCD-treated juices (≤7 h) was greatly shorter than in the untreated or heat-treated juices (= 60 h), but the difference in the precipitation formation time was not significant ($P > 0.05$) regardless of HPCD treatment time.

Figure 1 compares the PSD patterns of peach juices after HPCD and heat. The untreated juice showed a PSD from 0.3 to 12 μm with a volume peak at 0.954 μm. The d_{43} and d_{32} of the untreated juice were 2.15 ± 0.01 and 1.24 ± 0.01 μm (**Table 1**), respectively. The difference between d_{43} and d_{32} indicated that the untreated peach juice was very polydisperse. The PSD pattern of the heat-treated juice was changed with the highest volume peak at 0.869 μm. In addition, the PSD pattern of the heat-treated juice was wider than that of the untreated juice; two smaller volume peaks at larger particle size were shown, which was possibly due to heat-induced protein coagulation as depicted in the work of Reiter et al. (*22*). However, the d_{43} and d_{32} values for the untreated and heat-treated juice show no significant difference in **Table 1**.

As shown in **Figure 1**, the PSD patterns were significantly changed after HPCD. All of the HPCD-treated juices showed the widest PSD patterns from 0.3 to 600 μm with three volume peaks, and the highest volume peak was shifted to 18−21 μm. The particle size of the HPCD-treated juices significantly increased as compared with the untreated and heat-treated juices. The volume value of the HPCD-treated juices all showed a noticeable decrease for the smaller particles and an increase for the larger particles. With increasing treatment time, the volume peak values showed an increase for the smaller particles and a decrease for the larger particles; meanwhile, the particle sizes of the highest volume peak showed a left shift. It was suggested that the PSD patterns after HPCD exhibited an evolution to smaller particle sizes with increasing treatment time. For the HPCD-treated juices, the volumes of the larger particles between 3 and 600 μm were 90.32, 83.02, 75.45, and 75.84%, respectively, which decreased with increasing treatment time. **Table 1** shows that d_{43} and d_{32} of the HPCD-treated juices were both significantly larger than those of the untreated and heat-treated juices and exhibited a decrease

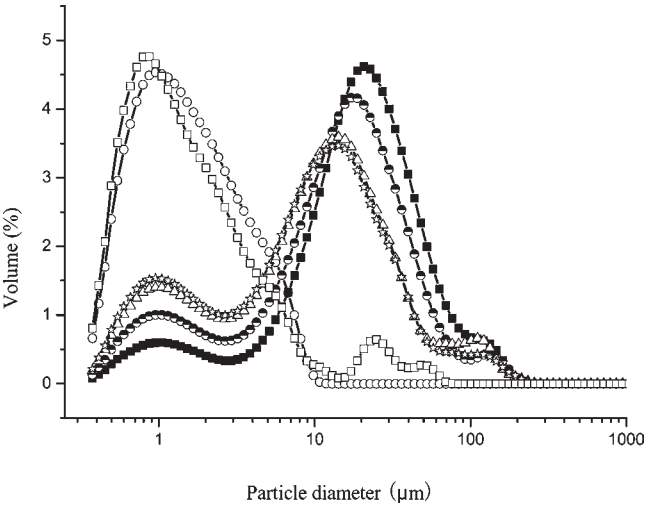

Figure 1. PSD patterns of peach juices treated by HPCD at 30 MPa and 55 °C and heat at 90 °C for 1 min: (○) untreated; (■) 30 s; (◓) 10 min; (△) 40 min; (☆) 60 min; (□) heat treatment.

with increasing treatment time. The alteration of the PSD patterns of the HPCD-treated juices was consistent with a previous study dealing with carrot juice (*13*).

Thus, acceleration of the precipitation formation (**Table 1**) almost concurred with the increase of juice particle size in the HPCD-treated juices (**Figure 1**), suggesting that increasing juice particle size induced the acceleration of precipitation formation, because bigger particles in juices may settle due to gravity (*1*).

pH and ζ-Potential Analysis. In the present work, no effects of HPCD and heat could be found on pH and ζ-potential of peach juices (**Table 1**). The values of the ζ-potential were negative and kept constant around −6.7 mV. Croak et al. (*23*) noted that the cloud particle may be surrounded with a protective coating of negatively charged pectin, resulting in an overall negative surface charge. It should be noted that the above ζ-potential and pH were measured after decompression. In fact, the pH of the system could be significantly reduced by the carbonic acid from the dissolved CO_2 during the HPCD processing (*24*). Balaban et al. (*10*) showed that the initial pH value of orange juice could be reduced from 3.8 to 3.1 during HPCD, but increased again to the initial value due to depressurization. Park et al. (*14*) showed that the pH of the carrot juice could be reduced from 6.5 to 4.4 at 4.90 MPa even after HPCD; similar results were also reported in the work of Zhou et al. (*13*). On the basis of mathematical models, Meyssami (*25*) calculated that the pH of an ascorbic acid−citric acid−water simulation system decreased from 3.8 to 3.2, closely following experimentally measured pH values. Croak (*23*) reported that the absolute value of ζ-potential in orange juice decreased with decreasing pH. Therefore, it was reasonable to believe that the pH and the absolute value of ζ-potential in the peach juice could be lower than 3.8 and 6.7 during HPCD, respectively, and these declines possibly induced the coagulation of protein in

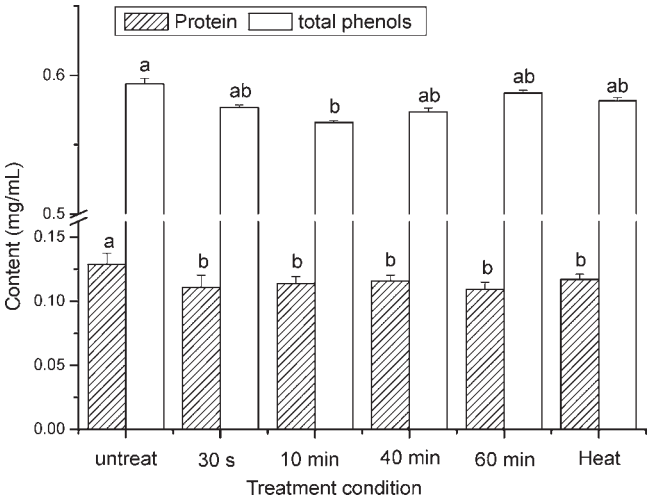

Figure 2. Protein and total phenols in the sera of peach juices treated by HPCD at 30 MPa and 55 °C for 30 s, 10 min, 40 min, 60 min, and heat at 90 °C for 1 min.

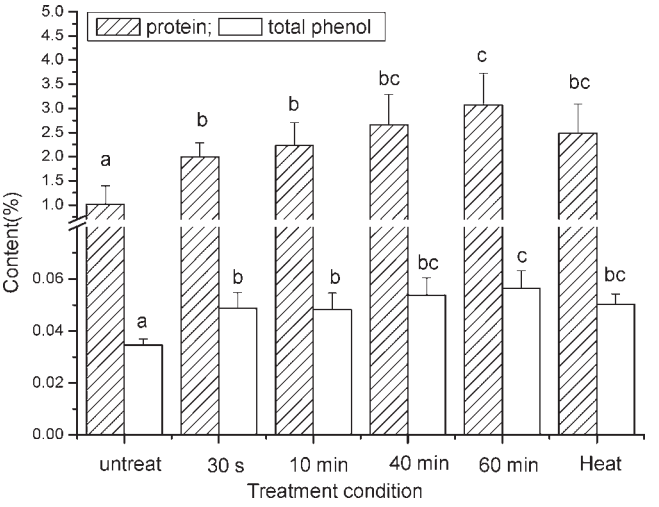

Figure 3. Protein and total phenols in the dry precipitations o peach juices treated by HPCD at 30 MPa and 55 °C for 30 s, 10 min, 40 min, 60 min, and heat at 90 °C for 1 min.

the juices, responsible for the alteration of the PSD patterns and the acceleration of precipitation formation in the HPCD-treated juices.

Analysis of Protein and Total Phenols in Juice Serum and Precipitation. To verify the coagulation of protein induced by HPCD, the protein content was measured in the juice sera and the precipitation of the juices. As shown in **Figures 2** and **3**, a significant decrease of the protein content was observed in the HPCD- and heat-treated juice sera, corresponding to their significant increase in the precipitation. Because casein could be precipitated by H_2CO_3 formed by reaction between CO_2 and water in milk, HPCD has been used for protein production in a continuous process instead of organic acids as precipitants used to isolate casein from milk (*26*). HPCD also has been applied to precipitate soy protein, which was suitable for the effective precipitation of soy proteins and prevents local pH overshoot (*27*). Thus, the decrease of the protein content in the HPCD-treated juice sera was mostly attributed to the pH and the absolute value of ζ-potential decline inducing the coagulation of protein, whereas the decrease of the protein content in the heat-treated juice serum was mostly attributed to heat-coagulated protein. Proteins and polyphenolic compounds can combine to form soluble complexes; these can grow to colloidal size and larger still, which can lead to sediment (*28*). Thus, the total phenols content was also measured. A declining trend or significant decline of the total phenols content was obtained in the HPCD- and heat-treated juice sera, corresponding to their significant increase in the precipitation (**Figures 2** and **3**). The decrease of the protein and the decline of the polyphenols in the HPCD- and heat-treated juice sera were similar, but the above-mentioned alteration of the PSD patterns (**Figure 1**) and the acceleration of the precipitation formation (**Table 1**) in the HPCD-treated juice were not observed in the heat-treated juice. This disagreement indicated that the mechanism of the alteration of the PSD patterns and the precipitation formation induced by HPCD and heat were probably different. It was hypothesized that an interaction between the HPCD-induced protein coagulation and the HPCD-induced homogenization effect, the HPCD-induced protein coagulation seemed to dominate for shorter treatment time and the HPCD-induced homogenization effect alternated for longer treatment time (*13*). The HPCD-induced homogenization effect resulted from the explosive action and the bubbling of CO_2 from the juices during the decompression.

Analysis of PME Activity and Contents of Calcium and Pectin. Pectin surrounding the cloud particles was attacked by PME, yielding acidic low-methoxy pectin, which could form insoluble pectate precipitates with polycations (*4*, *29*). Thus, the PME activity and the pectin and calcium in peach juices were determined in this study. **Figure 4** compares the PME activity and pectin content (normally represented by the content of the galacturonic acid) after HPCD and heat. As compared to the untreated peach juice, no significant variation of the PME activity could be observed after HPCD at 30 MPa and 55 °C regardless of the treatment time. However, there was a significant decrease in the PME activity of HPCD-treated peach juices after 10 min. Heat made no significant difference for inactivation effect of PME from HPCD for 40 and 60 min. The largest reduction of the PME activity induced by HPCD was 14% for 60 min; the PME activity by heat at 90 °C for 1 min was reduced only by 30% under the severe conditions in this study. The original PME in the juices was difficult to be effectively inactivated, which could be attributed to the fact that the PME was bound to the plant cell wall, which contains a lot of natural stabilizing factors (*16*, *30*).

As above-mentioned, the alteration of the PSD patterns and the acceleration of the precipitate formation in the HPCD-treated juices were observed (**Figure 1** and **Table 1**). Moreover, no significant variation of the PME activity could be observed after HPCD as compared with the untreated peach juice. Thus, the mechanism of HPCD on juice destabilization was a nonenzymatic action mode and not linked with PME activity. This observation was in agreement with a previous study. Kincal et al. (*31*) found no link between PME inactivation and cloud retention with the HPCD system. Croak et al. (*23*) reported that at a low pH of 2.5 cloud particles showed faster aggregation and higher particle size, possibly because of a decreased surface charge (*23*). The pH might play an important role in the aggregation of the HPCD-treated peach juices. On the basis of the above-discussed observation in this study, the pH of the HPCD-treated peach juices was indeed < 3.8 due to the dissociation of H_2CO_3 into H^+ during HPCD. Thus, the alteration of the PSD patterns and the acceleration of the precipitation formation in the HPCD-treated juices showed faster aggregation of cloud particles, also suggesting the decreased surface charge of cloud particles in an acidic environment caused by HPCD.

HPCD had no significant effects on the content of galacturonic acid (**Figure 4**) and calcium (**Table 1**). In fact, the activity of PME

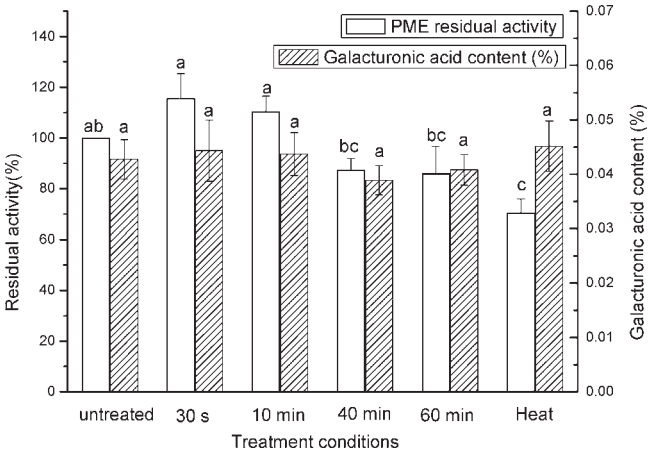

Figure 4. PME activity and pectin content of peach juices treated by HPCD at 30 MPa and 55 °C for 30 s, 10 min, 40 min, 60 min, and heat at 90 °C for 1 min.

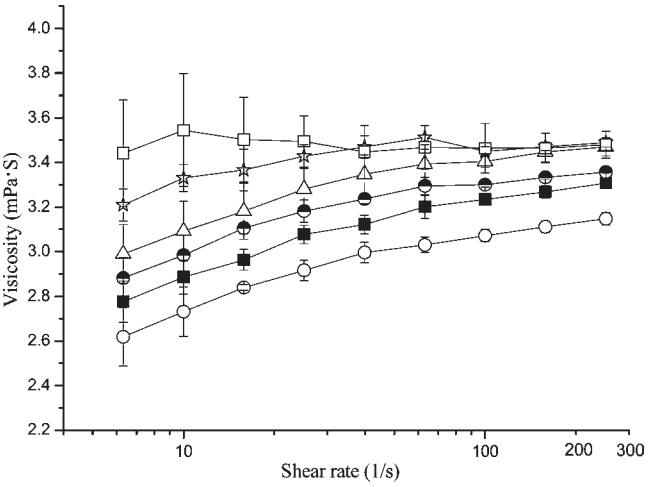

Figure 5. Viscosity of peach juices treated by HPCD at 30 MPa and 55 °C and heat at 90 °C for 1 min: (○) untreated; (■) 30 s; (◓) 10 min; (△) 40 min; (☆) 60 min; (□) heat treatment.

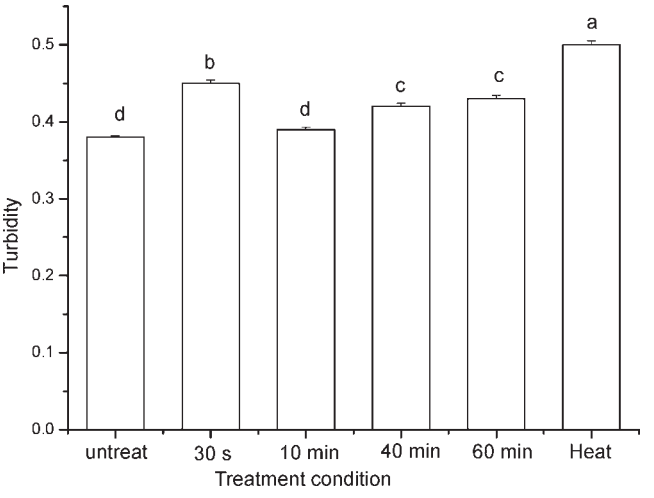

Figure 6. Turbidity of peach juice treated by HPCD at 30 MPa and 55 °C for 30 s, 10 min, 40 min, 60 min, and heat at 90 °C for 1 min.

with demethylation is present after treatments in this study, but the pectate was not formed. This was possibly due to the short time (≤60 min) of HPCD, because the required time for the precipitation formation in the untreated juice was 60 h in an enzymatic mode (**Table 1**). Moreover, PME activity in HPCD-treated juices at pH < 3.8 should be less than that at the actually determined value of pH 7.5 because the optimal pH of PME activity is near neutral and acidic pH decreases the PME activity (*32*).

Analysis of Flow Behavior and Viscosity. As shown in **Figure 5**, the viscosity in the untreated juice slightly increased with increasing shear rate (6−252 s^{-1}), exhibiting non-Newtonian characteristics of a dilatant fluid. The largest viscosity was obtained in the heat-treated juice, and the flow behavior was altered after heat. The viscosity in the HPCD-treated juices also increased with increasing the treatment time, and the same flow behavior was observed as compared with the untreated juice. Zhou et al. (*13*) showed that HPCD has a significant increase on the viscosity and does not change the Newtonian flow behavior of carrot juice. Most common fruit juices exhibited complex and variable rheological behavior, that is, time dependence, shear stress dependence, and viscoelasticity (*33*). Thus, the rheological properties of fruit juices appeared to be very much dependent on their varieties, state of ripening, concentration of juice/pulp and temperature variation (*34*), and processing conditions.

The increasing viscosity in heat- and HPCD-treated juice could be attributed to solubilization of pectin from cell walls in juices with higher temperatures by heat at 90 °C and HPCD at 55 °C. Moreover, particle interactions and particle size both play a role in determining the consistency (*35*). As we discussed above, the particle size of the HPCD-treated juices significantly increased as compared with the untreated and heat-treated juices. An increase in the number and/or intensity of interactions led to increased consistency, and pectin molecules were the main agents of physical interactions, whereas chemical interactions were dependent on many parameters (*35*). The increased viscosity by heat could positively promote the stabilization of peach juice, confirming that the precipitation formation time in the heat-treated juice was longer than that in the HPCD-treated juices (**Table 1**). Moreover, the precipitation formation time in the heat-treated juice was similar to that in the untreated juice, because a balance among the increased viscosity (**Figure 5**), the denatured protein (**Figure 3**) by heat, and PME action (**Figure 4**) occurred. According to the Stokes law, higher viscosity can better prevent the precipitation of particles in liquids (*36*). Although HPCD increased the viscosity of the juices, acceleration of the precipitate formation was observed as compared with the untreated and heat-treated juices (**Table 1**). These results indicate that the viscosity in the HPCD-treated juices played a lesser role in preventing precipitate formation.

Analysis of Juice Turbidity. Turbidity is considered to be a very important quality parameter in fresh unpasteurized juice (*14*). The effect of HPCD on juice turbidity is shown in **Figure 6**. The greatest turbidity was 0.50 for the heat-treated juice, significantly higher than that for the untreated and HPCD-treated juices. Although an abnormal result was found for the case at 10 min, HPCD could promote a significant increase of juice turbidity. HPCD led to the homogenization effect due to the explosive action and the bubbling of CO_2 from the juices during the decompression, which caused the formation of the smaller particles (*31*). This observation was in agreement with previous investigations. Arreola et al. (*15*) reported that turbidity is enhanced from 1.27 to 4.01 times by HPCD regardless of temperature and treatment time. Kincal et al. (*31*) also showed that the turbidity is increased between 446 and 836% after HPCD. It seemed that HPCD was an effective technique to preserve and even enhance juice turbidity. However, a contradictory result was reported by Park et al. (*14*) that turbidity is

greatly affected by HPCD, showing a 60% loss at 4.90 MPa, which was possibly due to a larger difference in parameters of HPCD (*13*).

The enhancement of turbidity by HPCD seemed to be contradictory to the acceleration of precipitate formation in peach juices. HPCD accelerated the aggregation of some larger particles due to the HPCD-induced protein coagulation in contrast with the untreated and heat-treated juices; the aggregation in HPCD-treated juices was easily removed, whereas unaggregated larger particles in the untreated and heat-treated juices were not removed by centrifugation. Meanwhile, the homogenization effect of HPCD caused smaller particles of the juice to colloid (*31*). Thus, the HPCD-treated juices contained more uniform particles than the untreated juices, and a higher turbidity was obtained.

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